A Research Proposal

3D BIOPRINTED BIOMIMETIC CORONARY ARTERY SCAFFOLDS FOR CARDIOVASCULAR TISSUE REGENERATION

Submitted to

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1 Introduction

Coronary artery disease (CAD) continues to be a major global health concern, impacting millions and leading to significant morbidity and mortality. It is caused by the narrowing or blockage of coronary arteries, which are essential blood channels that supply blood to the heart muscle. Plaque buildup in these arteries decreases blood flow, resulting in chest pain, heart attacks, and heart failure. While current CAD treatment options such as medication, angioplasty, stenting, and coronary artery bypass grafting have made significant advancements, they still have limits and adverse consequences such as restenosis, thrombosis, infection, and graft failure. Given these challenges, there is a need for innovative methods to improve the quality of life and survival of CAD patients.

This study aims to address this critical clinical need by developing a unique biomaterial specifically designed for coronary artery procedures. The primary objective is to develop a biomaterial that is not only biocompatible but also functional, which can be used to bioprint a scaffold for coronary artery that will replicate the physical properties of a coronary artery and will thus be physiologically relevant.

The project's goals are varied, including the design, fabrication, and validation of a biomaterial that mimics a healthy coronary artery in structure and mechanical capabilities. To achieve this, we have outlined the following comprehensive set of steps.

- **Decellularization:** Initially, the process of decellularization will be accomplished. It is a technique that involves the complete removal of cellular components from a coronary artery. This method leaves behind the extracellular matrix (ECM), which provides essential structural support and mechanical cues for the tissue.
- **Mechanical Benchmarking:** The next step involves measuring the mechanical properties of the decellularized coronary artery. We will assess factors such as stiffness, strength, and elasticity to establish a benchmark for our novel biomaterial.
- **Biomaterial Formulation:** Using natural and/or synthetic polymers, a biomaterial will be formulated, that can be processed into a hydrogel. This hydrogel is essential for bioprinting, a technology that allows for precise layer-by-layer fabrication.
- **Rheological Optimization:** For bioprinting it is essential to ensures that the biomaterial flows and deforms as required under various levels of stress. To prepare the selected biomaterial for bioprinting, the formulation will be further optimized based on the rheological analysis.

- **Bioprinting:** Leveraging the capabilities of bioprinting, we will create intricate 3D scaffolds. This technique allows us to deposit the biomaterial layer by layer, shaping it into the desired structure and size.
- **Mechanical Evaluation:** The mechanical properties of the bioprinted scaffolds will be subjected to in-depth evaluation. This includes assessments of stiffness, strength, and elasticity, all of which describes how the scaffolds respond to dynamic loading and stress relaxation. This is critical as the goal is to create a physiologically relevant coronary artery.
- **Optimization Through Additives:** To precisely replicate the mechanical properties of the native coronary artery, we will optimize the formulation of the 3D bioprinted scaffolds. Additives, such as crosslinking agents and/or nanoparticles, will be incorporated to enhance the mechanical performance of the scaffolds.
- ***In-vitro* Validation:** A basic *in-vitro* validation of the optimized formulation will be performed. This involves addition of relevant cells on the scaffolds, and a comprehensive assessment of their viability, proliferation, and differentiation.

This hypothesis is rooted in the belief that by using a decellularized coronary artery as a reference, a biomimetic coronary artery scaffolds can be engineered with similar mechanical properties. Furthermore, it is anticipated that the *in-vitro* validation will demonstrate that the scaffolds can support the growth and function of cardiovascular cells, emphasizing their potential for tissue regeneration.

As the world grapples with the pervasive burden of coronary artery disease, this research offers a ray of hope, promising to lead in a new era of treatment options and cardiovascular care. By exploring the uncharted field of biomaterials and tissue engineering, I aspire to bridge the gap between scientific innovation and clinical impact.

2 Literature Review

Tissue engineering is a multidisciplinary field that applies the principles of engineering and life sciences to develop biological substitutes that can restore, maintain, or improve tissue function. In the context of cardiovascular medicine, tissue engineering holds significant promise for addressing various cardiovascular diseases and conditions. It involves the development of engineered heart tissues, blood vessels, and cardiac patches using a combination of biomaterials, stem cells, and growth factors. These engineered tissues can be utilized for disease modeling, drug screening, and translational studies, thereby advancing the field of cardiovascular tissue engineering into the realm of precision medicine. The application of tissue engineering in cardiovascular medicine aims to repair the diseased heart, mitigate diabetes-related complications, and develop cardiac tissues for basic research and predictive toxicology applications. Furthermore, tissue engineering strategies have made remarkable progress in remodeling, replacing, and regenerating damaged cardiovascular tissues, offering potential solutions for congenital heart disease treatment.

The use of various cell types, including induced pluripotent stem cells and cardiovascular progenitor cells, in repopulating decellularized heart tissues demonstrates the potential of tissue engineering for personalized heart disease therapy. Additionally, the exploration of pericyte and cardiac stem cell secretome has unveiled new tactics for drug discovery in cardiovascular regenerative medicine, emphasizing the expanding research in this field. The development of injectable cardiac tissue engineering for the treatment of myocardial infarction highlights the potential of biomaterials to enhance cell-based therapies and improve their efficacy for cardiac disease. Moreover, the construction of whole intervertebral disc tissue engineering exemplifies the broader impact of tissue engineering techniques in regenerative medicine.

From a materials science perspective, heart disease can be viewed as a reduction in biomaterial performance, emphasizing the importance of biomaterials in cardiac tissue engineering. The utilization of umbilical cord cells as a source for cardiovascular tissue engineering further demonstrates the diverse cell sources being explored for tissue engineering applications in cardiovascular medicine. Overall, tissue engineering offers a promising approach for addressing the challenges in cardiovascular medicine, ranging from repairing damaged cardiac tissues to developing novel therapeutic interventions.

2.1 Challenges in current treatments for coronary artery diseases

The current treatments for coronary artery diseases present several challenges. Firstly, the pathogenic factors and pathological processes of coronary artery diseases, such as Kawasaki disease and spontaneous coronary artery dissection, are not entirely clear, which complicates the prevention and treatment of these conditions [1], [2]. Additionally, the treatment of Kawasaki disease in the acute phase aims to reduce inflammation in the coronary artery wall and prevent coronary thrombosis, while long-term therapy for individuals with coronary aneurysms focuses on preventing myocardial ischemia or infarction [3]. Moreover, the choice between percutaneous coronary intervention and coronary artery bypass graft as invasive treatment options for coronary artery disease presents its own set of challenges[4], [5]. Furthermore, the routine use of intravenous immunoglobulin treatment has reduced the incidence of coronary artery lesions in Kawasaki disease, but challenges remain in identifying predictors for coronary artery dilatation and intravenous immunoglobulin resistance [6]. Additionally, the management of patients with coronary stents undergoing non-cardiac surgery poses challenges, as guidelines recommend postponing elective surgery until completion of the recommended time of dual antiplatelet therapy after coronary artery stenting [7], [8]. Furthermore, the prevalence of severe coronary disease, such as left main or three-vessel coronary disease, is more common than generally perceived, and simple and low-cost tools may help in the selection of the most appropriate therapeutic approach [9]. Moreover, the failure to perform a coronary intervention when necessary, results in an accentuated increased risk of events and death, highlighting the challenges in timely and appropriate intervention for coronary artery disease [10]. Additionally, in the current practice, technical improvements of stent deployment and excellent results of guideline-directed optimal medical therapy have led to an increase in the patient population with moderate coronary artery disease, which could be treated with robotic coronary artery bypass grafting alone or in combination with stents, presenting new challenges in determining the most suitable treatment approach [11].

1.1. Role of 3D bioprinting in biomimetic coronary artery scaffolds

3D bioprinting is an innovative technology that has gained significant attention in the field of tissue engineering and regenerative medicine. It involves the precise layer-by-layer deposition of biomaterials and living cells to create complex 3D structures that mimic natural tissues and organs [12], [13]. This technology has shown promise in creating biomimetic coronary artery scaffolds, which are essential for addressing coronary artery disease and related conditions [14], [15]. The use of 3D bioprinting in tissue engineering applications offers several

advantages, including rapid fabrication, high precision, and the ability to customize the production of scaffolds [13], [16]. Furthermore, the development of 3D bioprinting has enabled the fabrication of novel 3D scaffolds with complex architectures, which is crucial for addressing the challenges associated with reliable and defined tissue regeneration [15], [16]. In the context of creating biomimetic coronary artery scaffolds, 3D bioprinting has the potential to revolutionize the field by providing a means to fabricate scaffolds with the desired properties, including the ability to mimic the native vasculature complex structure [15]. Additionally, the technology offers the ability to engineer highly controllable microenvironments, which is essential for creating functional and biomimetic coronary artery scaffolds [17]. The use of 3D bioprinting for vascularized tissue fabrication has been a subject of research, covering methods for bioprinting vascularized constructs and bioinks for vascularization, which are directly relevant to the creation of biomimetic coronary artery scaffolds [18]. The technology's ability to produce complex 3D structures to mimic the in vivo microenvironment is particularly relevant in the context of creating biomimetic coronary artery scaffolds [19].

2.2 Prevalence and impact of coronary artery disease on cardiovascular health

Coronary artery disease (CAD) is a prevalent and significant health concern, with a substantial impact on cardiovascular health. It is associated with a high prevalence of risk factors for cardiovascular disease, such as atherosclerosis, and is a leading cause of morbidity and mortality [20]. The presence and extent of vascular calcification have been shown to be significant predictors of cardiovascular morbidity and mortality in patients with end-stage renal disease, highlighting the importance of understanding and managing coronary artery calcification in this population [21]. In the general population, coronary artery calcium is strongly associated with the prevalence of cardiovascular disease, emphasizing the clinical relevance of coronary artery calcification as a marker of atherosclerotic burden and cardiovascular risk [22]. Furthermore, stable angina pectoris without obstructive coronary artery disease is associated with increased risks of major adverse cardiovascular events, indicating the need for comprehensive risk assessment and management strategies beyond obstructive CAD [23]. The global epidemiology of ischemic heart disease, including CAD, underscores its widespread impact, with a high prevalence, especially in the elderly population [24], [25]. Additionally, the prevalence of noncalcified plaques and coronary artery stenosis is an important consideration in patients with coronary calcium scores of zero, as it has implications for cardiovascular risk stratification and management [26]. Moreover, impaired

coronary flow reserve and coronary microvascular dysfunction are markers of adverse cardiovascular outcomes, acute coronary syndrome, and the development of heart failure, highlighting the multifaceted nature of CAD and its impact on cardiovascular health [27], [28]. The relationship between CAD and other cardiovascular conditions, such as aortic stenosis, rheumatic heart disease, and congenital heart disease, further emphasizes the interconnectedness of cardiovascular pathologies and the need for comprehensive management strategies that consider the diverse clinical presentations and risk factors associated with these conditions [20].

2.3 Existing treatment options for CAD

Each CAD condition must be treated differently. Even though there are plenty of treatment options, none of them can eradicate CAD completely. In the long run there might be a need arising for transplantation of the coronary artery through an open-heart surgery. This implies that the artery used should be extracted from the patient's own body or needs to be xenografted. While the former solution might not impact the immunity of the body, but in the latter case it is highly likely that the patient's body can reject the xenografted coronary artery. The alternative method is using the patient's own cells to construct a coronary artery that will resemble the one that is to be transplanted. This regenerative approach is possible through engineering a tissue for the specific application with precise physiological properties.

2.4 Principles of tissue engineering and its role in regenerative medicine

Tissue engineering is a multidisciplinary field that applies principles from engineering, biology, and medicine to promote the regeneration of diseased and injured tissues and organs [29]. It involves the process of replacing, engineering, or regenerating human cells, tissues, or organs to restore or establish normal function [30]. Stem cell research plays a leading role in regenerative medicine, which has evolved into an interdisciplinary field encompassing tissue engineering, gene therapy, biomaterials, and nanotechnology [31]. Tissue engineering follows the principles of cell transplantation, materials science, and engineering to develop biological substitutes that can restore and maintain normal function [32]. It focuses on the regeneration of neotissues from cells with the support of biomaterials and growth factors [33]. Regenerative medicine, including tissue engineering, has the potential to restore the function of native cell lines, grow new tissues or organs, model disease states, and enhance the viability of transplanted organs [34]. It is based on stem cell technology and tissue engineering to replace or regenerate human tissues and organs and restore their functions [31]. The ability to identify

and manipulate stem cells has significantly advanced regenerative medicine and contributed to the development of tissue engineering-based clinical therapies [35]. 3D bioprinting is a cutting-edge technology that holds promise in tissue engineering and regenerative medicine. It involves the precise layer-by-layer deposition of biomaterials and living cells to create complex tissue structures [36]. This technology offers a wide variety of possibilities and provides hope for treating untreatable diseases [36]. Furthermore, the application of 3D bioprinting in tissue engineering has the potential to overcome limitations in the clinical regeneration of ligaments or tendons [37]. In conclusion, tissue engineering, a key component of regenerative medicine, aims to restore and maintain normal function by replacing, engineering, or regenerating human cells, tissues, or organs. Stem cell research, biomaterials, and nanotechnology play crucial roles in this interdisciplinary field. 3D bioprinting, as a cutting-edge technology, offers promising prospects for creating complex tissue structures and addressing limitations in tissue regeneration.

2.5 Biomimetic design principles for creating functional and biocompatible coronary artery scaffolds

Biomimicry, the practice of emulating nature's designs and processes, has gained significant attention in tissue engineering for creating functional and biocompatible coronary artery scaffolds. Biomimetic design principles are essential for developing such scaffolds, as they aim to replicate the natural environment of the tissue. This involves achieving in-depth cell penetration, controlling pore sizes, biomechanical properties, and minimizing solvent toxicity [38]. Biomimicry properties have been harnessed in cartilage tissue engineering, demonstrating promising outcomes [39]. Strategic biomimicry is crucial for engineering tissue connectivity and scaffold design, emphasizing the importance of mimicking natural tissues at a multiscale level [40]. Furthermore, the use of biomimetic electrical stimulation has found important applications in engineering functional cardiac tissues, emphasizing the diverse applications of biomimicry in tissue engineering [41]. The evolution of coronary stent technology and the rationale for new generation platforms underscore the importance of biomimetic design in creating ideal coronary stent technology [42]. Biomimetic scaffold design with complex shapes and structures has become a focus with the advent of additive manufacturing, highlighting the role of advanced manufacturing techniques in achieving biomimicry in tissue engineering [43]. Additionally, the potential of bioresorbable scaffolds (BRSs) to overcome limitations and restore physiological conditions in treated vessels underscores the importance of biomimicry in scaffold design [44]. The scientific community has rapidly embraced the concept of

bioresorbable scaffolds, indicating the growing significance of biomimetic approaches in clinical practice [14]. Biomimetic principles have been established as guiding technical design considerations for tissue-engineered vascular networks, emphasizing the importance of mimicking natural vascular structures in scaffold design [45]. The fabrication and maturation of 3D tissue models within microfluidic platforms have substantially contributed to the engineering of tissues with high structural and functional biomimicry [46].

To date, 3D bioprinting has shown promising results in the field of cardiovascular tissue engineering, particularly in the context of coronary artery regeneration. Recent studies have highlighted the potential of 3D bioprinting in creating patient-specific models for cardiovascular diseases [47]. This technology has also been applied to visualize the proximal to mid portion of the coronary artery tree, indicating its relevance in the field of coronary artery imaging [48]. Furthermore, advancements in 3D bioprinting have led to the development of novel 3D scaffolds with complex architectures, addressing the challenges associated with reliable and defined tissue regeneration [16]. Additionally, the review by emphasized the current approaches for tissue and organ engineering, including scaffold designing and recent advances in 3D bioprinting, demonstrating the extended application of 3D bioprinting techniques in the medical field [49]. In the specific context of coronary artery regeneration, recent studies have focused on the application of 3D bioprinting in cardiac tissue engineering, indicating a growing interest in utilizing this technique for the development of new strategies for cardiac tissue regeneration [50]. These developments underscore the potential of 3D bioprinting in addressing the challenges associated with coronary artery regeneration.

2.6 Materials used for 3D bioprinting of coronary artery

The materials used for bioprinting coronary artery scaffolds are crucial for ensuring the success of tissue engineering and regenerative medicine applications. Various materials have been explored for this purpose, including hydrogels, polymers, and bioinks. Faulker-Jones et al. highlighted the potential of bioprinting human pluripotent stem cells (hPSCs) and their differentiation into hepatocyte-like cells using hydrogel and polyethylene glycol dimethacrylate [51]. Zhang et al. emphasized the precision offered by three-dimensional (3D) bioprinting in fabricating scaffolds, allowing the precise placement of cells, biochemical factors, and biomaterials [52]. Maina et al. discussed the limitations faced by vascular grafts bioprinted on scaffolds, indicating the challenges in traditional tissue engineering [53]. Yazdanpanah et al. provided an overview of the wide range of biomaterials, both natural and synthetic polymers, explored for scaffold bioprinting [54].

2.6.1 Collagen

The collagen superfamily comprises 28 members numbered with Roman numerals in vertebrates (I –XXVIII). Collagen possesses a major advantage in being physiologically relevant, biodegradable, biocompatible, and easily available. Out of this Type I, II, III, IV, V, VII, IX, X are commercially used as biomaterials in tissue engineering and regenerative medicine [55].

Class	**Types**	**Sources**[55]
Fibril-forming	I	Bone, skin, tendons, ligaments, cornea (represent 90% of total collagen of the human body)
	II	Cartilage, intervertebrate disc, notochord, vitreous humor in the eye
	III	Skin, blood vessels
	V	Bone, skin, tendons, ligaments, cornea
	XI	Cartilage, intervertebrate disc, notochord, vitreous humor in the eye
Network-forming	IV	Basal lamina
	VII	Beneath stratified squamous epithelia

Collagen can be extracted from various sources, including bovine, porcine by-products, and ovine tendon and skin. Bovine collagen is biocompatible and widely used for tendon reinforcement, hernia repair, skin and wound healing, and plastic surgery. Other sources include fish tissue, chicken, duck, and rabbit skin [55].

2.6.2 Gelatin

Gelatin or Gelatine is a type of insoluble protein produced by hydrolysis of collagen extracted from a variety of animal sources (porcine and bovine) such as skin, bones, and connective tissues. Gelatin is also derived from marine sources such as skins, bones, fins, heads, scales, or swim bladder.

The amino acid content of gelatin is almost identical in composition with the original collagen from which it was derived. It also has repeating sequences of Gly-X-Y, with Gly representing proline and Y representing hydroxyproline. Gelatin possesses all the essential amino acids, with the exception of tryptophan. It contains a high amount of glycine, proline, and hydroxyproline

but is low in cysteine, methionine, and tyrosine. Proline and hydroxyproline are very important with respect to gelling properties [56].

Gelatin is translucent, almost tasteless and odourless, brittle, easy to digest, and a nonallergenic protein. The colour of the powder depends on the gelatin source. For example, gelatin derived from cows and pigs possess a yellowish colour compared with gelatin derived from fish. Gelatin derived from pork skin also has less colour than gelatin derived from bone or hide. However, colour generally does not influence the properties of gelatin [56].

Gelatin can be classified into two types and this is determined by gelatin pre-treatment during the gelatin manufacturing process. Type A gelatin with an isoionic point of 6–9 is obtained from acid-treated collagen, whereas type B gelatin (isoionic point of 5) is derived from an alkali-treated precursor. Gelatin derived from pig skin is normally referred to as type A gelatin and gelatin derived from beef skin is referred to as type B gelatin. Gel strength and viscosity are the most important physical properties of gelatin. Gel strength, also known as ‘bloom’ value, is a measure of the strength and stiffness of the gelatin, reflecting the average molecular weight of its constituents, and is usually between 30 and 300 bloom (<150 is considered to be a low bloom, 150–220 a medium bloom, and 220–300 a high bloom). Higher bloom value indicates greater gelatin strength. Based on the type of product required and its function, a different bloom value for gelatin is applied [56].

Gelatin is commercially manufactured from collagen using a series of processing steps, including conditioning, extraction, filtration or purification, concentration, drying, and grinding. However, the process used may vary depending on the type of collagen source used, porcine or bovine sources, and skin or bone or hide origin. During the conditioning process, the material is cut and washed to cleanse and degrease the collagen before it is used to manufacture gelatin using either a type A (acid) or type B (alkaline) process [56].

2.6.3 Alginate

Biocompatibility, biodegradability, shear tinning behaviour, quick gelation and an easy crosslinking process makes alginate one of the most studied polysaccharides in the field of regenerative medicine.

The study utilized glycine–arginine– glycine aspartic acid–serine–proline(GRGDSP), modified alginate(RA) and unmodified alginate(UA) at varying concentrations and blending ratios with PEO poly to create uniform nanofibers, which are RA and UA were dissolved in

ultrapure deionized water (diH2O) at concentrations from 0.25 to 2% (w/v), PEO was dissolved in water at a concentration of 4.0% (w/v) and, blending ratios of alginate/PEO: 20:80, 40:60, 50:50, 60:40, 80:20. The electrospun scaffolds showed enhanced capacity for human dermal fibroblast cell attachment, spreading, and proliferation [57].

Chitin-calcium alginate composite fibres were created using shrimp shells and alginic acid, with a combination of Chitin 1.75% (w/v), alginate 0.58% (w/v). These fibres showed comparable stress values to calcium alginate fibres and absorption capacities. In a rat model, they outperformed chitin-covered wounds in wound healing, with complete dermal fibrosis coverage after 7 days. However, optimization is needed for enhanced healing properties [58].

Wet spinning techniques were used to create absorbent alginate fibres that contained sulphanilamide, an antibacterial and anti-inflammatory agent by mixing sulphanilamide with alginate solution of 3.5% (w/v). The mass ratios of sulphanilamide to sodium alginate in aqueous solution were 1% (w/v), 2% (w/v), 5% (w/v) and 10% (w/v). The fibres showed high loading content and efficient drug release, with enhanced efficacy compared to pure alginate fibres. This study suggests the potential of these fibres for wound dressing applications due to sustained drug release [59].

2.6.4 Laponite

Laponite is a nano silicate clay that can be used to prepare composites with other biomaterial thereby not only improving its mechanical properties but also its conductive properties which is important for developing a biomaterial for the coronary artery.

The study in biofabrication presents a novel nanoengineered ion-covalent cross-linkable bioink for 3D bioprinted organotypic tumour models. The bioink combines gelatin methacryloyl (GelMA) and synthetic nanosilicate (Laponite) to mimic the tumour extracellular matrix. The study evaluates the physical properties, printability, and cell viability of the composite hydrogels, finding that low GelMA (5%) with high Laponite (2.5%–3.5%) exhibits suitable mechanical properties for 3D printing [60].

This study tested the potential of Laponite, a gel-forming clay, combined with mafenide as a wound dressing material. Laponite/mafenide (Lap/Maf) hydrogel was formulated in three different ratios of Lap/ Maf 1:1, 1:2, 1:3. and the Lap/Maf/Alginate film was created by combining Lap/Maf gel (1:1) with alginate. The study found that mafenide molecules intercalated between Laponite nanoparticles, and the eluted mafenide showed active

antibacterial effects against all tested bacteria. The Lap/Maf composite has the potential to be used as an antibiotic eluting gel or film for wound healing [61].

This study presents the development of a hydrogel-based ink of Laponite and alginate for 3D printing. Five different ink gel compositions were prepared by keeping alginate concentration constant at 1% (w/v) but varying Laponite concentrations from 1.5 to 10% (w/v). It introduces a standard protocol for defining optimal printing parameters, providing new printability evaluation parameters. The study also demonstrates the stability of the constructs over time, allowing for future *in vitro or in vivo* developments. Finally, this study demonstrates the absence of cytotoxicity of the printed formulations, hence, setting the stage for their use in the field of biomedicine [62].

2.7 Mechanical testing of scaffolds

The mechanical properties of coronary artery scaffolds play a crucial role in successful tissue regeneration. The scaffolds need to possess suitable mechanical strength to provide support for cell differentiation, growth, and proliferation, as well as to ensure proper transfer of loads acting on the scaffolds to the adjacent host tissues during tissue regeneration.

Uniaxial testing is useful for comparative studies of diseased and healthy arterial tissue. Inflation testing offers better simulation of in-vivo geometry and loading conditions. Advanced mechanical testing techniques can enhance surgical simulations, predict risk, and model pathological conditions by analysing the dynamic remodelling of arterial tissue in response to its mechanical environment [63].

Assessing the mechanical properties of bioprinted tissues involves specific tests tailored to the tissue type's forces, such as compressive testing for bone and tensile strength for skin or ligaments. Integrating testing structures into tissue design, such as grip tabs and indentation testing, can improve tensile testing and non-destructive testing. Usability considerations for clinical end users, such as suture retention and mechanical matching, are also crucial [64].

This study reviews mechanical testing of articular cartilage and repair constructs over the past decade, identifying common configurations and key parameters. Compression testing was the most prevalent, followed by frictional properties evaluation. Variations in parameters such as strain rate, equilibrium time, and maximum strain were identified. Validation studies highlighted the need for standardized testing criteria, promoting robust evaluation of physical properties in novel treatment strategies for cartilage tissue engineers [65].

The integration of various cell sources with 3D bioprinting for coronary artery scaffolds is a critical area of research in tissue engineering. Several studies have investigated the combination of cells with biomaterials to create bioinks for 3D bioprinting, with the aim of fabricating vascularized constructs and tissue-engineered scaffolds. For example, Richards et al. discussed the methods of bioprinting vascularized constructs and bioinks for vascularization, highlighting recent innovations in 3D printing and biomaterials for the next generation of 3D bioprinting for vascularized tissue fabrication [18]. Additionally, Maina et al. presented their work on creating scaffold-free, bioreactor-free, 3D bioprinted vessels from a combination of rat vascular smooth muscle cells and fibroblasts, emphasizing the retention of tensile and mechanical strength of native vessels [53]. Furthermore, Tharakan et al. emphasized the emerging use of bioprinting stem cells into 3D scaffolds for regenerative medicine and bone tissue engineering [1].

The human heart is composed of following cell types:

- Cardiac Fibroblasts
- Cardiomyocytes
- Smooth Muscle Cells
- Endothelial Cells
- Adipocytes
- Mesothelial Cells
- Neuronal Cells
- Pericytes

3 Aim and Objective

3.1 Aim

To design, synthesize, and validate a novel biomaterial for coronary artery interventions.

3.2 Objectives

1. To decellularize a coronary artery from an animal
2. To evaluate the mechanical properties of a coronary artery from an animal
3. To formulate a suitable biomaterial
4. To optimize the selected biomaterial for 3D bioprinting
5. To create 3D scaffolds using 3D bioprinting
6. To evaluate the mechanical properties of the 3D bioprinted scaffolds

7. To perform an *in-vitro* validation of the formulation.

4 Proposed Hypothesis

We hypothesize that by benchmarking a decellularized coronary artery with a carefully formulated biomaterial, optimized through additive incorporation and 3D bioprinting technology, it is possible to engineer biomimetic coronary artery scaffolds. These scaffolds, when subjected to in-depth mechanical evaluation and iterative optimization, will exhibit mechanical properties comparable to native coronary arteries. Moreover, the *in-vitro* validation of the optimized formulation will confirm its potential for cardiovascular tissue regeneration.

A decellularized coronary artery from an animal will be subsequent evaluated for its mechanical properties to set the benchmark. Formulating a suitable biomaterial is crucial for mimicking the native coronary artery's structural and mechanical properties. Through meticulous selection and optimization of biomaterials, coupled with 3D bioprinting techniques, we aim to fabricate scaffolds with optimum fidelity. To ensure that the 3D bioprinted scaffolds meet the mechanical standards set by native coronary arteries, we propose iterative optimization. Additives will be incorporated into the formulation to enhance the scaffold's mechanical properties. The optimized formulation will undergo *in-vitro* validation to confirm its suitability for cardiovascular tissue regeneration.

5 Methodology

- Extensive literature survey
- Biomaterial selection and design according to coronary artery
- Biomaterial development
- Biomaterial characterization
- Preclinical validation

6 Importance of Study

Coronary artery disease is a leading global cause of morbidity and mortality, necessitating innovative approaches to improve patient outcomes. This research can significantly enhance patient safety, minimize restenosis, and extend the durability of coronary interventions.

Moreover, it contributes to precision medicine by tailoring treatment options, aligns with the principles of patient-centered care, and has the potential to reduce the public health burden associated with coronary artery disease. Cardiovascular tissue engineering holds immense

potential for regenerating damaged heart tissue. In India, researchers are actively contributing to this field, focusing on innovative biomaterial development for 3D bioprinting.

6.1 National Status

The first study [67] explored hybrid scaffolds incorporating conductive nanoparticles for cardiac tissue engineering. The researchers, using GelMA hydrogels with carbon-based nanoparticles, found that electrically conductive scaffolds significantly promoted desirable cardiomyocyte morphology and functional cardiac marker expression. The study emphasizes the importance of engineered cardiac tissues' maturity and functionality, highlighting the role of conductive substrates in promoting mechanotransduction.

Another study [68] investigated the use of extracellular vesicles (EVs) derived from human adipose-derived mesenchymal stem cells for enhancing vascular scaffold remodeling. In vivo experiments in rats showed that EV-functionalized vascular grafts demonstrated improved patency and increased elastin and collagen deposition compared to cell-seeded and blank controls. This work supports the potential of EVs as a cell-free therapeutic base for vascular scaffolds, offering a safer alternative to cell-based technologies.

Yet another research [69] focused on 3D bioprinting of vascularized cardiac patches using a non-mulberry silk-based conductive bioink with carbon nanotubes. The study demonstrated the bioink's ability to enhance cardiomyocyte viability, sarcomere formation, and beating rate. Incorporating oxygen-releasing and immunomodulatory microspheres further extended the constructs' viability in a hypoxic environment. In vivo implantation revealed improved host neovascularization and implant acceptance, emphasizing the importance of modulating the post-ischemic microenvironment.

In a unique approach, researchers developed a 3D cardiac tissue construct using stacked silk-cell monolayers [70]. Utilizing both mulberry and non-mulberry silk fibroin, the study showcased the potential of non-mulberry silk as a biomaterial for cardiac tissue regeneration. The constructed 3D cardiac tissue demonstrated structural integrity, uniform cellular distribution, and promising prospects for further preclinical investigations.

Researchers introduced an innovative 3D printing-based framework for fabricating self-supporting tubular structures using decellularized extracellular matrix (dECM) hydrogel [71]. The study demonstrated improved mechanical stability within seven days of culture and highlighted the potential of this approach for fabricating tubular organs with

mechanocompromised hydrogels. This innovative strategy aligns with the broader goal of developing tissue-specific hydrogels for hollow organ engineering.

Indian researchers are making significant strides in biomaterial development for 3D bioprinting of cardiovascular tissue. The studies discussed here contribute valuable insights into the design of hybrid scaffolds, the use of extracellular vesicles, and the development of innovative bioinks and frameworks. These efforts collectively advance the national status of cardiovascular tissue engineering in India, offering promising avenues for future clinical applications.

6.2 International Status

The bioprinting of cardiomyocytes relies on the utilization of biomaterials such as hydrogels, conductive materials, and biocompatible polymers to create scaffolds that provide the necessary microenvironment for cell growth, maturation, and regeneration. Hydrogels are widely used as cell-laden materials for bioprinting due to their biocompatibility[72]. Additionally, conductive biomaterials such as carbon- and gold-based nanomaterials, conductive polymers, and ceramics have shown promise in cardiac tissue engineering due to their electrical conductivity and ability to promote cell signaling transduction[73]. Biomaterials offer advantages in cardiac tissue engineering by providing biophysical and biochemical cues critical for cell maturation and regeneration[74]. The use of soft materials like hydrogels and biocompatible polymers in 3D printing techniques, also known as bioprinting, allows for the printing of cells with hydrogel inks, facilitating the creation of biomimetic scaffolds for tissue engineering[75]. Furthermore, the fabrication of 3D scaffolds using biomaterials and cell seeding, as well as the simultaneous printing of biomaterials and cells, are approaches used in scaffold-based bioprinting for cardiac tissue engineering[76]. 3D-printed cardiac scaffolds composed of polymeric and cellular components have been shown to satisfy the requirements of cardiac tissue regeneration, offering customizable geometric morphology and mechanical support for injured tissue[77]. Cationic silica nanoparticles have been exploited for bioprinting large-scale constructs with high printing fidelity, enabling the fabrication of 3D structures containing living cells with matched 3D shape and architecture[78]. Moreover, the use of plant-derived biomaterials has shown potential in cardiac tissue repair, serving various applications such as scaffolds and bioink in 3D biofabrication[79].

In the context of cardiovascular tissue engineering, the use of biomaterials in 3D bioprinting has shown promise in the development of personalized in vitro disease models, high-throughput assays, and stem cell therapies[80]. Additionally, the application of hydrogels as

bioinks has been widely recognized for their cross-linking capability to create scaffold structures for tissue engineering applications[81]. The development of biomaterial formulations with specific biochemical characteristics and physical properties suitable for bioprinting of 3D scaffolds is a pivotal challenge in tissue engineering[82]. Moreover, the review by Charbe et al. highlights the current advancements and future prospects for 3D bio-printing in engineering ex vivo cardiac tissue and associated vasculature, such as coronary arteries, emphasizing the importance of biomaterial selection in achieving functional cardiovascular tissues[83]. The study also emphasizes the need for biomaterials that can ensure the safe and efficacious process of seeding cells, biomolecules, and biomaterials using 3D bioprinting, while addressing limitations such as cost, volumetric bioprinting, integrity, and strength of biomaterials[84].

In summary, the bioprinting of cardiovascular tissues, including arteries, relies heavily on the selection of appropriate biomaterials. Collagen, alginate, gelatin, hyaluronic acid, deECM scaffolds, and hydrogels have been identified as key biomaterials used in 3D bioprinting for cardiovascular tissue engineering. These biomaterials offer biocompatibility, structural mimicry, and the potential for creating functional vascular constructs.

7 Research Work Plan

S. No.	Milestone	Work Elements	Months
			3 6 9 12 15 18 21 24 27 30 33 36 39 42 45
1	Literature Review	1. To study the anatomy of coronary artery, specifically for an animal.	
		2. To find the methods for decellularization	
		3. To understand the characterization techniques and related methodologies to confirm the decellularization of coronary artery	
		4. To find methodologies for testing the mechanical properties of the decellularized coronary artery	
		5. To understand the process and characterization techniques required for optimization of bioprinting parameters that are required for 3D bioprinting	
2	To decellularize a coronary artery from an animal	1. Collection of coronary arteries from an animal slaughterhouse	
		2. To decellularize the coronary artery	
		3. Confirmation of decellularization	
3	To evaluate the mechanical properties of a coronary artery from an animal	1. Axial stress Strain Relation	
		2. Tensile Strength	
		3. Youngs Modulus	
4		1. Selection of suitable biomaterial	

	To formulate a suitable biomaterial	2. Rheological studies of the biomaterial																
		3. Selection and optimization of respective crosslinker																
		4. Perform degradation studies																
5	To optimize the selected biomaterial for 3D bioprinting and creating 3D scaffolds using 3D bioprinting. (Rheology)	1. Optimization of extrusion width																
		2. Optimization of extrusion rate																
		3. Evaluation of extrusion delay																
		4. Evaluation of layer height																
		5. Optimization of infill density																
6	To evaluate the mechanical properties of the 3D bioprinted scaffolds.	1. To optimize the formulation using additives to meet the mechanical properties of the coronary artery																
7	To perform an in-vitro validation of the formulation.	1. Cell culture																
		2. Cell Proliferation (Live/Dead assay)																

8 References

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